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A COMPARISON OF THE NUTRITIONAL VALUE OF NATIVE AND INTRODUCED GRASSES FOR WINTER PASTURE

W. J. Pigden

Department of Plant Science
University of Alberta

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UNIVERSITY OF ALBERTA

FACULTY OF AGRICULTURE
DEPARTMENT OF PLANT SCIENCE

The undersigned hereby certify that they have read and recommend to the School of Graduate Studies for acceptance, a thesis entitled, "A Comparison of the Nutritional Value of Native and Introduced Grasses for Winter Pasture", submitted by Wallace James Pigden, B.S.A., in partial fulfilment of the requirements for the degree of Master of Science.

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THE UNIVERSITY OF ALBERTA

A COMPARISON OF THE NUTRITIONAL VALUE OF NATIVE AND INTRODUCED GRASSES FOR WINTER PASTURE

A DISSERTATION
SUBMITTED TO THE SCHOOL OF GRADUATE STUDIES
IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE
OF MASTER OF SCIENCE

FACULTY OF AGRICULTURE

by
Wallace James Pigden

EDMONTON, ALBERTA
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A COMPARISON OF THE NUTRITIONAL VALUE OF NATIVE AND INTRODUCED GRASSES FOR WINTER PASTURE

W. J. Pigden

INTRODUCTION

The livestock industry is an important branch of Agriculture in the three Prairie Provinces, Manitoba, Saskatchewan, and Alberta. Figures supplied by the 1948-49 Canada Year Book, indicate that the livestock population numbered some 3,944,000 cattle, 1,080,000 sheep, and 1,114,000 horses, in 1947. According to Clark and Tisdale (7), the major portion of the forage requirements for these animals is supplied by native pasture lands which have been estimated to occupy at least forty million acres.

A considerable proportion of this acreage is utilized for its cured forage during the fall and winter months. This is possible for two main reasons:

(1) The warm chinook winds which usually partially clear the snow from the pastures and moderate the temperatures in many areas.

(2) Several grasses common to the prairies of western Canada exhibit to a marked degree a property arbitrarily designated as "curing". Curing is defined as the process which conditions the plant so that after growth ceases it:

(a) retains a relatively high nutritive value during late summer and throughout the subsequent fall and early winter,

(b) preserves its physical form, stems and leaves do not become excessively hardened, neither do they lacerate or decompose to any extent for some 8 to 10 months after the curing process occurs. Curing normally takes place during late July but may be as early as mid June, or as late as the latter part of August, depending on the season.

Ranchers report that curing results in a forage which maintains sheep and cattle in a thrifty condition during the winter with little or no supplemental feeding, despite the fact that protein content of the cured forage is usually less than five per cent (7). Hence, from the viewpoint of the rancher, curing is an invaluable asset - it decreases production costs by shortening materially the winter feeding period.

As stated previously, the bulk of the forage for livestock production on the Prairie is derived from native grass

pastures. An increasing acreage of unproductive land - depleted pastures, submarginal cultivated lands and abandoned farms - is however, being regrassed with comparatively high yielding introduced grasses (6).

Several of these grasses, now being extensively used for this purpose, appear to lack, wholly or in part, the curing property. This deficiency tends to limit their usefulness in a production program where feed reserves are normally accumulated in the form of winter pasture. Therefore it would be highly desirable to obtain a grass which would incorporate the curing property - as exemplified by the most valuable native grasses - with high yield, winter hardiness, drought resistance and other desirable characteristics displayed by certain cultivated species.

The first logical step in the development of such a superior grass appears to be a study of the factor or factors involved in the curing process. Once this knowledge has been obtained, the plant breeder can decide whether the information can be applied to the development of improved types of forage for winter pasture.

The following native grass species are credited with the ability to cure to a greater or lesser degree:

	Common Name	Scientific Name
1.	Common speargrass	*Stipa comata* Trin. and Rup.
2.	Blue grama grass	*Bouteloua gracilis* (H.B.K.) Lag.
3.	Western wheatgrass	*Agropyron Smithii* Rydb.
4.	Rough fescue	*Festuca scabrella* Torr.
5.	Parry's oatgrass	*Danthonia Parryi* Scribn.
6.	Short awned porcupine grass	*Stipa spartea var. Curtiseta* Hitchc.
7.	Niggerwool	*Carex filifolia* Nutt.
8.	June grass	*Koleria cristata* Pers.
9.	Northern wheatgrass	*Agropyron dasystachym* (Hook) Scribn.

In addition several grass species of minor importance as well as certain forbes, particularly salt sage, *Atriptex nuttallii* S. Wats. and winterfat, *Eurotia lanata* (Pursh) Moq., may have this property.

These species are dominant in the prairie area within the provinces of Manitoba, Saskatchewan and Alberta. The foothill area on the west and as far north and east as the southern boundary of the Park Belt is included within the region of distribution.

Preliminary investigations were started in 1948 at the Dominion Experimental Station, Swift Current, Saskatchewan. Two native grasses (known to cure well) and two cultivated grasses (non-curing, or to a limited degree) were selected for comparative studies. Specimens of each grass were collected, mounted and photographed.

A comprehensive literature survey was carried out at the University of Alberta, 1948 - 49, to determine what investigational methods would be followed. As a result it was decided to study the problem from both a biochemical and histological standpoint with special reference to lignin.

The biochemical analysis was designed to lend support to, or disprove, two main hypotheses:

1. Lignin content:

   Since lignin is almost indigestible, a high lignin content would probably markedly lower the nutritive value of cured forage.

2. Solubility of reserve carbohydrates:

   A readily water soluble reserve substance such as sucrose - commonly found in the Gramineae - would be leached out of the dried tissues much more readily than a less soluble carbohydrate reserve such as starch.

The histological study was intended to supply a general overall picture of the anatomy of leaf and stem, with special reference to the position and extent of the lignified tissues. It was hoped to determine whether any relation existed between these features and the curing habit.

Most of the histological study was carried out at the Dominion Experimental Station, Swift Current, during the summer of 1949. The biochemical analyses were also started there in the Soils Laboratory and were completed in the laboratories of the Department of Plant Science, University of Alberta, 1949-50.

LITERATURE REVIEW

Insofar as the writer is aware, there are no published data on studies dealing with the curing ability of the native grasses of western Canada. This literature survey is therefore of necessity based on information derived from more indirect sources, mainly in the fields of animal nutrition and plant physiology.

Clark and Tisdale (7) have reported the chemical analysis of approximately 1,000 samples of native vegetation from the area concerned. Data on the chemical composition of the most important cultivated species within this area are also available (7, 32). Unfortunately this information, as supplied by standard chemical feedstuffs analysis, does not show differences of sufficient magnitude to account for the superior nutritive value of cured native grass forage as compared with cultivated grasses. This is not surprising, however, as many of our leading animal nutritionists have repeatedly demonstrated serious shortcomings in the present system of feedingstuffs analysis, particularly the division of the carbohydrates into crude fibre and nitrogen-free extract (Weende Method), (43).

Investigations have shown that frequently much of the lignin, a relatively indigestible constituent of feeds, is included with the nitrogen-free extract, the supposedly readily digestible fraction (46, 47). Conversely, crude fibre, which is normally considered to be the woody, poorly digested portion, may have a coefficient of digestibility fully equal to that of the nitrogen-free extract (11, 47).

Substitution of the crude fibre and nitrogen-free extract proximate analysis by determinations for lignin, cellulose and other carbohydrates, has been advocated by Crampton and Maynard (11). For research purposes, particularly where digestibility trials are involved, many investigators now carry out parallel determinations using both methods. Most of the evidence from such trials indicates that the results using the proposed modification agree much more closely with actual digestibility studies than do those using the Weende Method (9, 10, 11, 20, 27, 35, 39) hence it seems likely that it would provide a more accurate index of the actual nutritional value of a given feed.

Since the development and use of these improved analytical techniques, lignin has been recognized as an increasingly important factor in determining the utilization of forages. For this reason it is dealt with here, in detail.

The chemical structure of lignin is still obscure. Although it is included in the carbohydrate fraction in the routine feedstuffs analysis, it is not a true carbohydrate since it is known to be an aromatic compound containing methoxyl groups (2). Whatever its structure may be, its main significance as a constituent of feeds lies in its relatively indigestible nature.

The digestion coefficients for lignin vary considerably depending on species of animal concerned, species of plant and chemical method employed for its isolation (10, 11, 13, 21, 22, 24, 37, 42, 57, 58). Most of these coefficients fall within the range from zero to 20 per cent, but Bondi and Meyer (1) have claimed the unusually high values of 35.1 to 64.0 per cent for the digestibility of lignin in various Egyptian forages. Most of the more recent evidence, however, supports the contention that lignin is relatively indigestible, so much so that Forbes and Garrigus (22) have found it to be a useful marker for computing the dry matter intake of grazing animals. In any event, according to Crampton (8) the small amounts of lignin which are digested are of little dietary value to the animal since most of it is excreted in the urine.

Numerous research workers have indicated that the digestibility of feedstuffs is directly related to their lignin content. Crampton (9) and Crampton and Forshaw (10) carried out digestibility

trials with rabbits using pasture herbage clipped at various stages of maturity. They found that the lowest growth rates and poorest digestibilities coincided with the herbage samples highest in lignin. Forbes and Garrigus (22) using steers, found dry matter digestibility and total digestible nutrient content of various forages varied inversely with lignin content of the forage. Drapala et al (15) used sheep to test the digestibility of several cuttings of red clover at various growth stages. These workers concluded on the basis of their chemical analyses, that the substantial decreases in digestibility coefficients which took place between cuttings two and four could only be accounted for by small increases in lignin content. The digestibility of crested wheatgrass cut at various stages of maturity was tested in Montana with Hereford steer calves (4). The results indicated that highest lignin values were associated with lowest gains in weight. Lancaster (33) found a very high negative correlation of -.98 between lignin content of 14 feeds (hays and succulents) and the digestibility of their organic matter by sheep. Phillips and Loughlin (53) found a high negative regression between per cent lignin and digestibility for alfalfa and timothy hays.

Because pasture grasses have a relatively high lignin content depending on stage of growth, species, climatic conditions and other factors, lignin is of particular interest to the pasture

biochemist. A large number of investigations have established the fact that in general lignin content of forage plants increases steadily with advancing maturity. Patton and Gieseker (51) found that the lignin content of five Montana grasses from two localities increased steadily as the season advanced from about five per cent in May, to 18 per cent in September. The following season, Patton (50) reported a similar relationship for grasses from the same plantings and four additional species as well. The only exception was glue grama grass (Bouteloua gracilis) which had a surprisingly high lignin content at an early vegetative stage. Drapale _et_ _al_ (15) reported the lignin content of red clover for two seasons ranged from 7.3 per cent to 15.2 per cent for the first and fifth cuttings respectively. Other workers in this field, Crampton (9), Crampton and Forshaw (10), and Norman (48), have all published similar findings for numerous grasses, legumes and mixed herbages.

That wide variations exist in lignin content between species and between varieties is also known. According to Patton and Gieseker (51), mountain bromegrass (_Bromus_ _marginatus_) Nees. and Fairway crested wheatgrass (_Agropyron_ _cristatum_) (L.) Gaertn., have a much lower lignin content than smooth bromegrass (_Bromus_ _inermis_) Leyss., and Standard crested wheatgrass respectively.

Lignin content of forages is also influenced by environmental factors which adversely affect growth. Patton and Gieseker (51) showed that grasses from drier regions of Montana reached

their maximum lignin content earlier in the season. Maynard (41) reviewed the findings of several workers in this regard and reported that unusually dry or cold weather apparently causes abnormal increases in lignin content at earlier growth stages.

Important as the quantitative influence of lignin on digestibility appears to be, there is good reason to believe that still more important principles may be involved. Cramptom and Forshaw (10) noted a marked decrease in the digestibility of pasture herbage over the short period of ten days with only small increases in lignin content. These workers suggest that at a certain stage of growth, mode of deposition in plant tissues may be more important than the actual increase in lignin content. Evidence to support this assumption is not entirely lacking. Drapala _et al_ (15) applied histological methods to a study of the process of lignification in red clover and made the following observations:

1. Lignification increases steadily with advancing maturity starting at the base of the plant, and proceeding upward.
2. The regions around the vascular bundles are mainly involved, the xylem itself becoming more heavily lignified and sclerenchyma tissue forming in the immediate area.
3. Xylem parenchyma underwent lignification at maturity thus making the food reserves in these cells partially or wholly unavailable to the animal.

Additional information was gained on the behaviour of lignified tissues in the alimentary tract of the sheep by studying slides prepared from feces residues (15). These slides indicated that the general increase in the number and size of the pieces of undigested plant tissue was directly related to higher lignin content and advancing maturity of the red clover forage.

These important observations indicate that most of the lignin, in some forages at least, up to a certain stage of maturity is deposited mainly in the vascular bundles. At this point lignin gradually begins to infiltrate and encrust the walls of adjacent tissues and a marked drop in the digestibility of the forage is likely to occur.

The detailed manner in which increasing lignification affects the digestibility of forages is believed to be as follows: As the cell wall develops and thickens, lignin and hemicellulose are deposited along with cellulose to give interpenetrating systems (48), the lignin acting as a physical barrier to prevent the decomposition of cellulose (41, 48). Unless the cellulose cell wall is broken down, it in turn seems likely to hinder the action of enzymes on the more digestible nutrients such as protein, fat, starch, etc. (41, 48).

Irrespective of the degree to which lignin reduces the availability of the readily digestible nutrients, its adverse

effects on the utilization of cellulose as such, is an extremely important factor. The value of cellulose as a feedstuff may be realized when we consider that in mature forages it comprises nearly one-half of the total carbohydrates which make up 60 - 85 per cent of the dry weight of the plant (41). The breakdown of plant cellulose to simpler products is accomplished by the microorganisms which inhabit the alimentary tract of herbivorous animals. Although cellulose cannot be expected to provide the animal with as much absorbable energy per pound as is provided by the enzymic digestion of starch, because of the loss of gases in the fermentation process (40), it does provide a large part of the energy derived from roughages.

Several European workers have shown that the digestibility of coarse roughages is greatly enhanced by partial removal of the lignin, which can be accomplished by a pretreatment with dilute alkali. Ferguson (21) concluded that alkali treatment of good wheat straw approximately doubled its feeding value to the ruminant. Homb (29) claims that treatment with sodium hydroxide, increased the coefficient of digestibility of the organic matter of straw from 42.4 to 63.7. Watson (59) reports that in England during the last war considerable amounts of wheat and barley straw were treated in

this manner to improve their digestibility.

Cellulose in a relatively pure form such as that isolated from plants is claimed to be highly digestible by ruminants (35, 36, 38). On the other hand, cellulose associated with lignin and other substances in forages is believed to be digested to a much smaller extent (21, 35, 36, 38). According to Norman and Fuller (49) "since lignification is a process of maturity, there are concurrent changes in composition which must be taken into account, such as a reduction in protein content and in water-soluble constituents. When due allowance is made for these, however, it is found that the cellulose of a material containing five per cent lignin is far more easily attacked than the cellulose of a material containing fifteen per cent."

Other factors which have a definite bearing on the availability of plant nutrients in cured forage are soluble carbohydrates such as sugars, protein, and ether extract. Each of these is briefly discussed in turn.

According to Guilbert and Mead (25) range cattle in the California foothills fatten readily on bur clover immediately after it cures on the stem. They also observed that if heavy rains occurred at this stage the high nutritive content of this plant was frequently reduced to a sub-maintenance level within

a few days. Subsequent experiments indicated that significant decreases in digestibility of the cured forage occurred when the bur clover was exposed to sunlight and rain. These workers also found that as much as 35 per cent of the nitrogen-free extract and up to 16 per cent of the crude protein could be removed from bur clover by artificial leaching in the laboratory (26). Norman (48) has reported that as much as 34 per cent of a water soluble fructosan occurred in rye grass *Lolium italicium* var. Western Wolths, in England at an early growth stage. Morosov (45), working in Russia, found over 20 per cent of inulin-like substances presumably fructosan in the stems of smooth bromegrass at a mature stage. Le Clerc and Breazeale (34) experimented with wheat, oats, and various other plants and reported that the largest losses of soluble nutrients by leaching occurred when the plants reached maturity. These data suggest that considerable losses in nutrients, especially carbohydrates, may occur if they are present in a water soluble form at the mature stage.

Translocation of soluble reserves from the aerial parts of the plant to the roots late in the season may also be a factor. Rapp (54) found that Johnson grass stored most of its carbohydrate reserves in the stem during the growing season as sucrose and transferred it to the roots at maturity.

Since the protein content of forages almost invariably declines with advancing maturity a part of the usual decrease in digestibility which occurs as the season progresses can undoubtedly be attributed to this factor. This important aspect of the field of nutrition will not be discussed here; rather it is desired to call attention to the very low protein content of cured native grasses (7), which obviously contributes relatively little towards the maintenance requirements of the range animal. The popular concept has been that rations consisting of roughages with a very wide nutritive ratio require a protein supplement for efficient digestion of the roughage (40). There is some evidence to suggest that the protein requirement for efficient roughage utilization is extremely low unless starch or starchy grains are also included in the ration (3).

According to Louw et al (36) old dry winter grazing in South Africa with about three per cent protein, is apparently not deficient in nutrients required by ruminal organisms responsible for the breakdown of cellulose to a limit presumably set by the degree of lignification.

The digestibility and energy value of the ether extract (crude fat) fraction of forages is usually fairly low

in comparison to the ether extract of seeds (40). This is because the extracting solvent removes from the plant material, in addition to the true fats, i.e. esters of fatty acids, chlorophyll, xanthophyll, carotene, waxes and other ether soluble substances which may amount to from 25 to 40 per cent of the total ether extract of leaf material (40). As a group, these non-lipid materials are very low in digestibility and tend to depress the digestibility of the ether extract as a whole (27, 40).

Several factors that are believed to influence the nutritive value of cured forages have been reviewed. Lignin is believed to be the most important.

MATERIALS AND METHODS

Grasses Studied

Since this investigation was regarded as being more or less of a preliminary nature, the grasses were selected to represent a fairly wide range of grass types found under various growing conditions. In this manner it was hoped to gain general information which would be of value for planning future investigations. The following grasses were chosen for comparative studies.

Native

(1) Common speargrass *Stipa comata* Trin. and Rup.

(2) Rough fescue *Festuca scabrella* Torr.

Cultivated

(1) Crested wheatgrass (Standard variety) *Agropyron cristatum* (L.) Beauv.

(2) Common or smooth bromegrass *Bromus inermis* Leyss.

These four grasses are illustrated (Figs. 1 to 4) and described briefly on the next four pages.

In addition, two grasses of minor importance found on the common speargrass site, namely sandgrass _Calamovilfa longifolia_ (Hook) Scribn. and Indian ricegrass _Oryzopsis hymenoides_ (R. and S.) Ricker were included in the histological study but not in the chemical analyses.

Hereafter the grasses will be referred to in the text as speargrass, fescue, crested wheatgrass, bromegrass, sandgrass, and ricegrass.

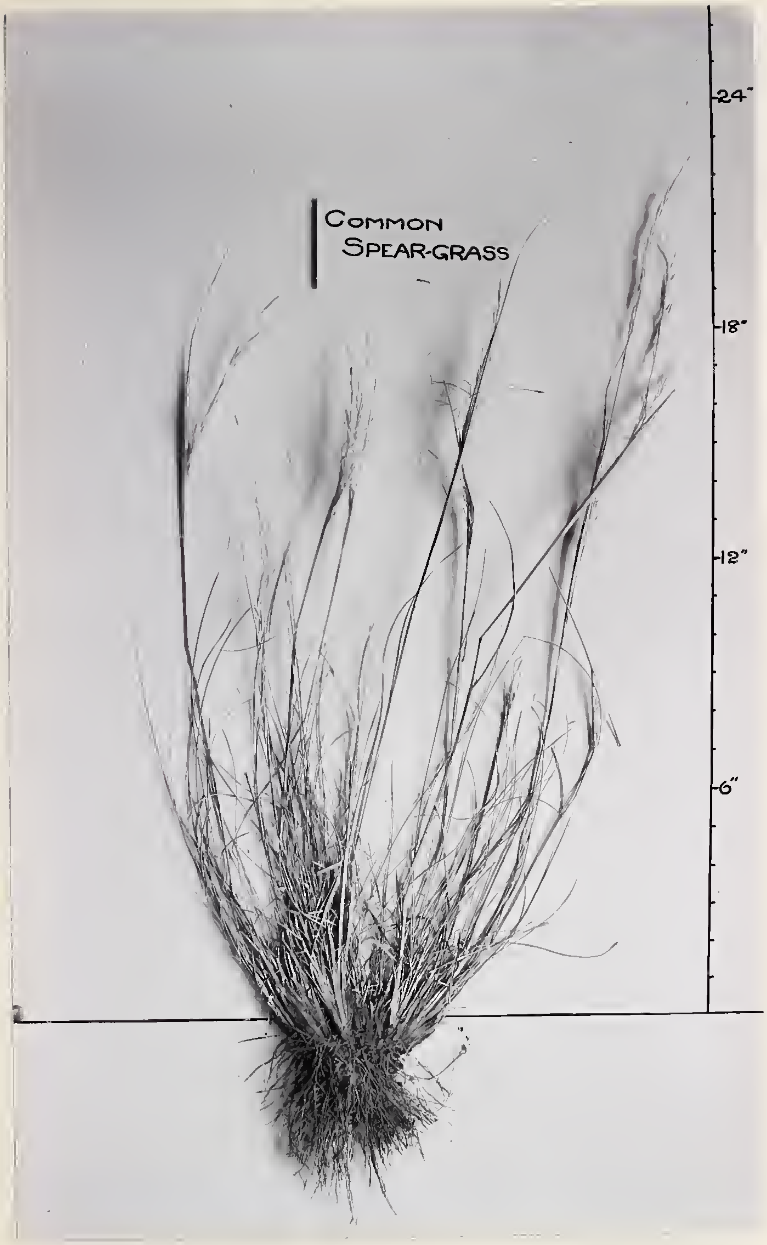

Fig. 1

Common speargrass

Stipa comata (native)

Speargrass is an extremely xerophytic grass, the second dominant in the shortgrass prairie and the dominant species in the mixed prairie association (5). Because of its relative abundance, palatability, and marked ability to cure, it is very highly regarded as a winter forage in the Prairie region.

This specimen shows greater than average production of seed stalks both in number and vigor. It is estimated that during a normal year at least 75 per cent of the cured forage (dry weight), would consist of leaves, mainly basal leaves. Frequently the ratio of leaf to stem is considerably higher as was the case in 1949, Table 1.

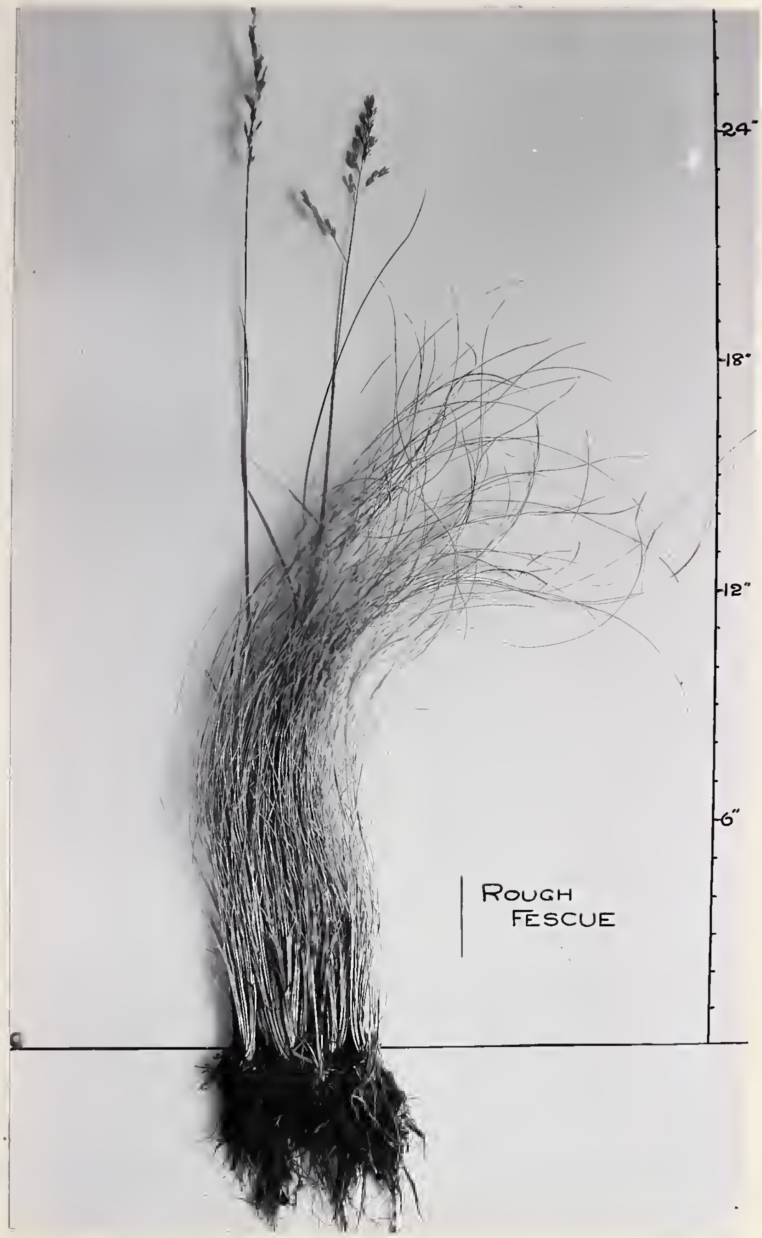

Fig. 2

Rough fescue

Festuca scabrella

(native)

Rough fescue is a less xerophytic grass than speargrass favoring a cooler climate with better moisture conditions. For this reason it is found only in favored locations in the mixed prairie area around Swift Current. It is the dominant species in the submontaine prairie association (5), producing an abundance of excellent cured forage in the Cypress Hills and Rocky Mountain Foothills. Seed stalks are usually produced very sparsely so basal leaves normally account for approximately 95 per cent or more of the dry weight of the plant. The cured leaves deteriorate very slowly and may be found in a good state of preservation up to 12 months or more after curing occurs.

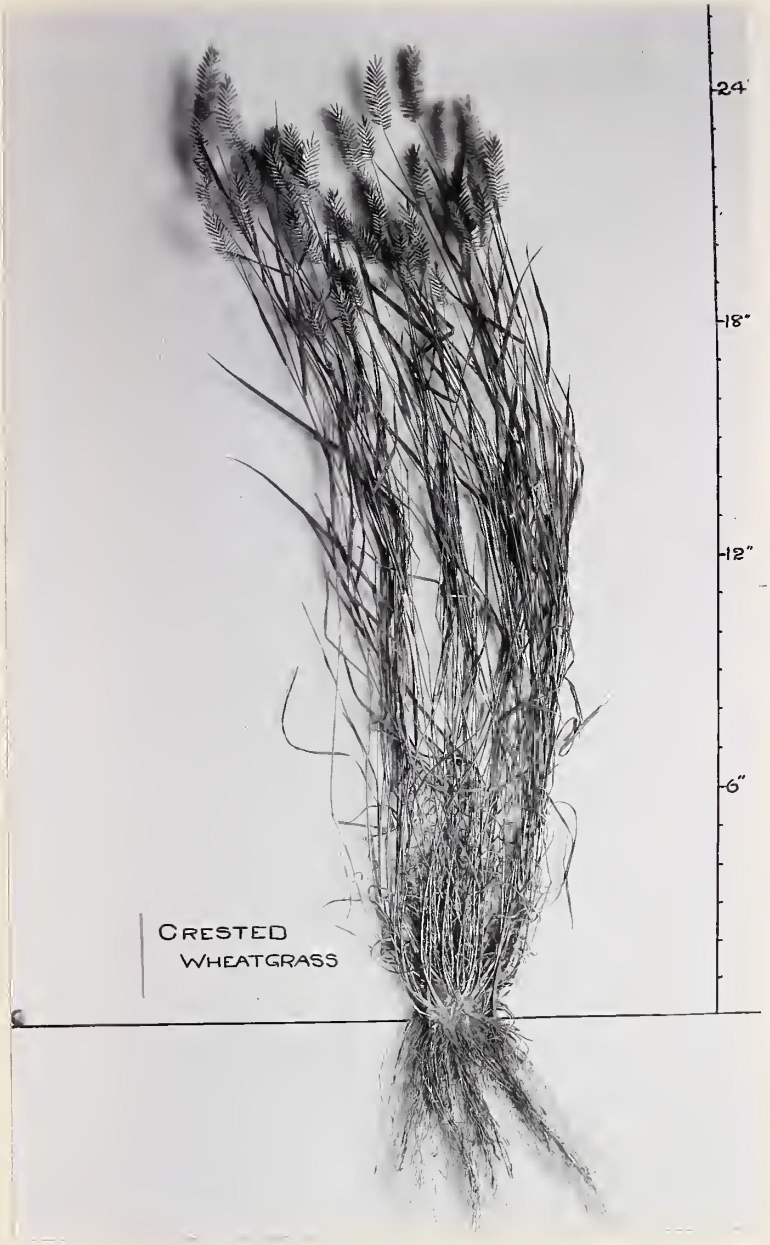

Fig. 3

Crested wheatgrass

Agropyron cristatum

(cultivated)

This is an introduced grass from the arid steppes of Russia and western Siberia. Because of its drought resistance, winter hardiness, and ability to compete successfully with most weeds, it has been used very extensively in regrassing programs in the dryland areas of the Prairie Provinces, (6). According to Kirk and Stevenson (32), it produces very palatable and highly nutritious forage for either hay or pasture; even the straw of crested wheatgrass, which has been harvested for seed, is readily eaten. Nevertheless, it is considered inferior to speargrass or fescue as a stem-cured forage. Many of the stem leaves break off during the fall and early winter but the stems resist weathering quite well and constitute most of the cured forage, Table 1.

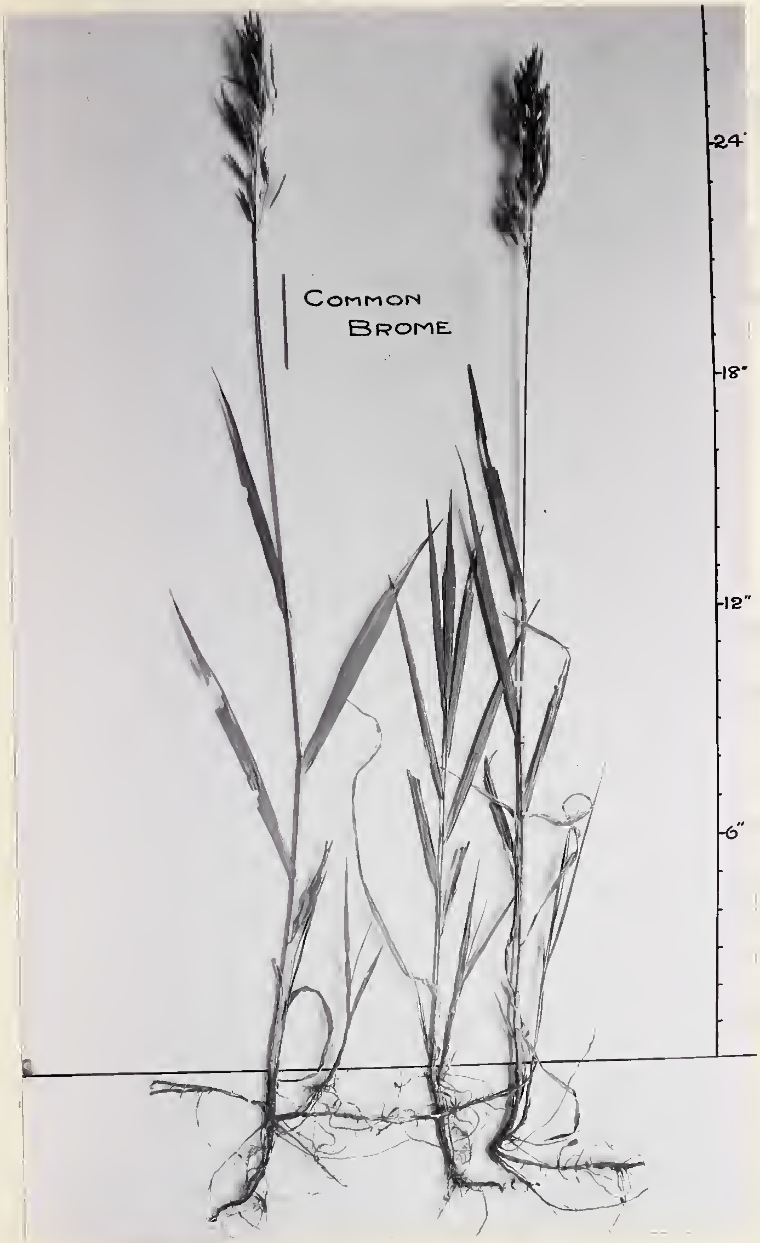

Fig. 4

Common or Smooth bromegrass

Bromus inermis

(cultivated)

Brome is also an introduced grass, being native to Europe, Siberia, and China. It is not as winter hardy or as drought resistant as crested wheatgrass, although it stands more drought than most of our cultivated grasses (30). For this reason it is not recommended for the dryland areas of the West, but it is used considerably for regrassing where moisture conditions are more favorable, such as occur in the Cypress Hills area (6). Where it is well adapted to the climate, it is known as an outstanding producer of excellent hay and pasture. As a stem cured forage, it is considered to be of very little nutritional value to the range animal and distinctly inferior to the three grasses previously discussed. During the early fall and winter months, under Swift Current conditions, practically all of the stem leaves and some of the stems, of the mature plants, break off and blow away.

Sites (Location and Description)

All sites were located within the mixed prairie area in the Swift Current district. Each one occupied about three-quarters of an acre.

1. Speargrass

This site was located on the east side of a low sand dune within an experimental exclosure of the Webb Research Area, 16 miles west of Swift Current. Sub-dominant grasses on this area were sandgrass, *Calamovilfa longifolia* (Hook) Scribn., and Indian ricegrass, *Oryzopsis hymenoides* (R. and S.) Ricker., with a few shrubs, wolf willow, *Elaeagnus commutata* Bernk., and chokecherry *Prunus melanocarpa* (A. Nels) Rydb., scattered over the lower third of the area. A few other grasses and forbes of minor importance were also found within the area.

2. Fescue

The fescue site was located on a fairly steep northern slope, within the Alexander Coulee Research Area, approximately six miles east of Swift Current. The slope was characterized by almost a pure stand of fescue - all other species being distinctly of minor importance - surrounded by a typical mixed prairie association.

3. Crested wheatgrass

The crested wheatgrass site was located on a level area in the south-west corner of the Swift Current airfield, approximately four miles east of Swift Current. Other grasses on the area were western wheatgrass, Agropyron Smithii Rydb., and wild barley, Hordeum jubatum L.

4. Brome

The bromegrass site was located in the south-west corner of a pasture field at the intersection of highways 1 and 4, within the boundaries of the Experimental Station proper. There was a relatively pure stand of bromegrass with some crested wheatgrass and a few other Agropyrons scattered over the sampling area.

Sampling Methods

1. Establishment of Quadrats

To assure that a representative sample was obtained from each site, two base lines were established at right angles to each other along two sides of each experimental area. The site was then mapped out on paper and divided into numbered

square meter units. Twelve of these meter quadrats were selected at random. Each quadrat was established within the area by measuring inward from the two base lines; the corners were marked with spikes. The quadrats were again sub-divided into quarters, a different quarter being cut at each stage of growth. Not all of the quarter quadrats contained the species being sampled but never less than eight samples were obtained from each site.

Note: Owing to the extremely dry weather, there was little early spring growth on the native grass sites. To obtain a large enough sample at Stage 1, fescue and speargrass were sampled anywhere within the experimental area.

2. Growth Stages

In order to study changes which occur as the plant develops and matures - information which might be essential for the interpretation of the data from the cured stage - the grasses were sampled at several stages of growth. Growth stages were preferred to definite dates so that proper comparisons could be made between species as the season progressed.

The five stages employed by Patton (50) for lignin and cellulose studies in Montana were chosen. They are as follows:

(1) Early leaf growth.

(2) Flowering culms in full bloom.

(3) Seeds ripe.

(4) Cured (most of the seeds fallen, plants dried on the stem).

(5) Remnants of the previous year's growth, (sampled in early spring). The fifth growth stage was considered relatively unimportant, so only a few analyses and tests were carried out for special purposes.

Hereafter, growth stages will be referred to by number, or as cured (Stage 4).

3. Harvesting

Because of diurnal variations known to exist in the chemical composition of plants (44), the grasses were sampled in the afternoon, usually between 2:00 p.m. and 5:00 p.m. They were cut with shears, approximately one inch above the crown, except for fescue which was cut at the top of the rough basal growth. The old growth was separated from the current year's

growth and discarded. Samples were divided into leaf and stem leaving the leaf sheath on the stem. Stem leaves of speargrass were included with basal leaves.

According to Gibbs (23) the leaf sheath of Blue panicum (_Panicum antidotale_) Retz., resembles the leaf more closely in chemical composition than it does the stem. This is probably true of other grasses as well. However, most of the leaves on the stems of brome and to a lesser extent of crested wheatgrass, break off and blow away after the plant dries in the early fall leaving a biological unit which consists mainly of stem and leaf sheaths. Conversely, the major part of the native grasses in the cured stage consists of basal leaf, Table 1. Thus a natural division exists which appears suitable for animal nutrition studies because most of the dry matter obtained by the grazing animal is considered in each case.

To make sure that the ripe seeds would not interfere with the qualitative starch test at Stage 3, the heads of the cultivated grasses were analysed separately. The speargrass heads are difficult to separate from the leaf sheath so they were left with the stem but the ripe seeds were removed. Residual seeds for all grasses at Stage 4 were removed for the same reason.

The stem of fescue was not analysed at any stage because very few culms were found within the sampling area.

4. Size of Sample

The quantity of forage dried for chemical analysis was limited to approximately 500 grams (fresh weight), by the size of the drying units. Accordingly, in some cases, the sample from the individual quarter quadrat was re-sampled to obtain a smaller unit, which was included in a composite sample for drying.

Drying the Samples

Lignin and soluble carbohydrate determinations were believed to be of fundamental importance when this investigation was started.

MacDougall and DeLong (37) showed that initial drying temperature had a direct influence on the apparent lignin content of plant materials; higher drying temperatures - within

the range studied - gave increased values for lignin. Furthermore certain carbohydrates readily change from one form to another when plant material is heat-dried and caramelization may occur at temperatures high enough to inactivate enzymes. Obviously then, drying by heat was undesirable.

Ellis et al (20) recommended air drying for plant materials where subsequent lignin determinations are to be made. However, it is well known that plants allowed to dry slowly, lose dry weight because of the cell respiratory activities. Dexter (14) showed that alfalfa hay air-dried in the mow over a period of three weeks lost a considerable portion of its sugar although whether these losses were caused by respiration or transformations was not known. While the error in lignin determinations caused by this factor would be small, the loss of sugars could introduce a rather large error into a soluble carbohydrate estimation. For this reason, air-drying was also considered unsatisfactory.

The lyophylization technique of Davies et al (12) is claimed to be capable of removing moisture from small and medium sized forage samples with minimal changes occurring in chemical composition. It was selected as the most suitable method available for drying the grass samples.

A brief general description of the method used by Davies and co-workers with slight modifications in equipment is as follows:

A closed insulated container enclosing a smaller unit, which was surrounded by powdered dry ice (solid CO_2), was transported to the sampling site. As the grass was harvested it was dropped into the inner container where it was rapidly frozen. Both containers were now closed, and in this state the sample would remain frozen for several hours or days, depending on the quantity of dry ice, efficiency of insulation, air temperatures, and other factors.

For the present studies the Kilner jar and Thermos flask used by Davies _et_ _al_ (12) was replaced by a one gallon insulated ice cream pack (available from dairy supply houses). The inner container was merely a tall metal can, the type sometimes used for packing and shipping hydrogen peroxide. When placed inside the ice cream pack there was ample room around it for a charge of dry ice.

The sample was taken to the laboratory, and transferred while still frozen, to a large, round-bottomed flask (5 liter). The flask was attached at once to a high vacuum system, and evacuated by an efficient oil pump. The vacuum unit consisted essentially of a cooled condenser immersed in a dry ice acetone bath at approximately -78°C., with a vacuum

lead-off to the pump through the condenser.

The Thermos flask used as a container for the dry ice acetone mixture which chilled the condenser was replaced by an ordinary one-half gallon can insulated on the outside with a layer of shredded paper 2 - 3 inches thick. While the Thermos flasks used by the authors would undoubtedly be more economical in utilization of dry ice, the two alternates described above served the purpose quite well.

Under vacuum the ice is sublimed out of the frozen plant tissues and refrozen on the inside of the condenser. The forage remains frozen until dry, a period of 5 - 6 hours being required to reduce the moisture content to approximately 0.5 per cent (12). By substituting a charge of phosphorus pentoxide for the condenser, the moisture content can be further reduced if so desired.

Difficulties encountered while using the freeze dry method were mainly concerned with insufficient vacuum. A Cenco Hyvac pump in good condition, rated by the manufacturer as capable of developing a vacuum of 0.0004 m.m. mercury, was found to be satisfactory. Several other pumps, either with a lower potential vacuum or else in poor condition, gave very disappointing results.

It was also found that the thick brome stems took about twice as long to dry as did leafy material, in some cases up to 12 hours was required. A method of shredding thick material before freezing it would speed up drying operations considerably.

Experience gained while using the freeze dry method indicates that it is quite suitable for drying a small number of samples for specialized research purposes. In its present form it appears to be too time- and labor-consuming for routine procedures where large numbers of samples are involved.

Grinding and Storing

The samples were ground in a small Gallenkamp hammer mill, so that 95 per cent would pass through an 80 mesh screen. They were stored in air-tight containers, (half pint sealers with rubber rings).

Because of the difficulties involved in keeping the ground material at a moisture free basis while grinding and carrying out the chemical analyses over an extended period, it

was allowed to return to a moisture content approaching that of air-dried material. Moisture content was determined periodically but there was little change from these values. According to the observations of Ekelund (18) there should be very little storage losses at this moisture content.

Biochemical Determinations

1. Lignin

The 72 per cent H_2SO_4 method of Ellis *et al* (20) was adopted and used as published. This method has proved useful and is being used quite extensively in nutritional work. Recent studies sponsored by the A.O.A.C. (19) indicate that determinations made using this method show a reasonably satisfactory degree of reproductibility both within and between laboratories.

2. Cellulose

The choice of a suitable method was difficult as there is no general agreement on a satisfactory method for nutritional

studies. This situation is well illustrated by Hallsworth's (28) critical comparison of seven different methods for cellulose determinations on feedstuffs. He found that the cellulose obtained by any one method varied widely in both amount and composition of the isolated product.

The modified Norman Jenkins method developed by Matrone et al (39) has been used frequently in nutritional studies since 1946. It is claimed to have an advantage over most methods for nutritional studies in that it isolates a biological unit which includes not only the cellulose, but also much of the so called cellulosans (pentosans and other materials associated with the "true" cellulose in the cell wall), substances which appear to be similar in degree of digestibility to the hexosan cellulose (21). This appears to be an advantage for nutritional work. Unfortunately however, studies sponsored by the A.O.A.C. (19) have indicated that this method is unsatisfactory in its present form.

Histological observations early in this investigation indicated that a considerable part of the native grass leaf consisted of cellulose fibres presumably in a relatively pure form. A method that would isolate primarily hexosan cellulose and thus supply a quantitative estimate of the amounts present seemed desirable. The Dichromate method of Druce and Willcox (16) appeared to fit this criterion most closely so it was used. It

has the admitted disadvantage of being so recent that insofar as the writer is aware, no data other than that supplied by the authors (17) are available to check their results.

The method was modified slightly. Filter sticks were substituted for the poplin Buchner funnel method of filtration and Gooch crucibles with asbestos mat were used for ashing instead of the silica crucibles specified by the authors. Use of the filter sticks permitted all filtering and washing operations to be carried out in one beaker, thus eliminating transfers.

3. Ether Extract (Crude fat)

Crude fat analyses were carried out by the official A.O.A.C. (43) procedure. Since most feed laboratories now substitute petroleum ether for ethyl ether as the extracting solvent, the method was modified to this extent.

4. Protein

The standard A.O.A.C. (43) Kjeldahl method for determining protein (total nitrogen x 6.25) was used, with the addition of one-quarter part of selenium in addition to the regular salt

mixture. The Boric acid modification of Scales and Harrison (56) was used in the distillation procedure.

5. Starch

A qualitative method was used to check the cured forage and determine whether starch was present or not. The method used by Ekelund (18) to test Swedish hays for starch appeared to be particularly well suited for this purpose, so it was used. Since the method is very simple and requires little space to describe it is outlined here.

Duplicate one-gram samples of ground forage were weighed out and placed in two large test tubes. Ten mgm. of wheat flour were added to one for a check. Both samples were boiled in a water bath for two minutes, cooled and 0.5 ml. solution of iodine (2 gm. I_2 + 6 gm. KI in 100 ml. water) was added. If the intensity of the color of the test solution did not not approach or exceed that of the check it was considered that there was less than 1% of starch present in the sample.

Histological Techniques

1. General

A typical leaf and stem were selected for sectioning from each quadrat on every sampling site, at all growth stages. Leaves, whether basal or stem, were sectioned at a point halfway between leaf tip and leaf base. In the case of stem leaves the second leaf from the top of the stem was used. Stems, including the leaf sheaths, were sectioned at a point approximately halfway between the two uppermost nodes. Rough fescue has no stem nodes so it was sectioned halfway between panicle and stem base.

A free hand method of sectioning was chosen so that microchemical tests could be applied to fresh tissue.

2. Microchemical Tests

(1) Lignin

The hydrochloric acid, phloroglucin method of Johannson (31) was applied to cross-sections of leaf or stem at all stages of growth.

(2) Fat

The Sudan IV method as outlined by Johannson (31) was used on cross-sections of the native leaves at Stages 4 and 5.

3. Sectioning Technique

Twelve pieces of leaf or stem to be sectioned, were laid out in order on the table. Freehand sections were cut from each with a sharp razor blade, while holding the material between two pieces of pith, keeping both blade and pith saturated with 70 per cent ethyl alcohol. The sections were dropped into a dish containing 70 per cent ethyl alcohol and left from 30 - 60 minutes, to dissolve out the chlorophyll. This last step was found to be essential to obtain a sharp, clear differentiation of tissues when the lignin test was applied.

4. Staining

After the chlorophyll had been removed, the sections were transferred to a slide and stained according to the particular test desired.

5. Photomicrography

After the microchemical test had been applied, the sections were examined under low power using a small student model microscope. Several of the most uniform and representative of the group were changed to a clean slide, mounted in a

glycerol water solution and photomicrographed.

The use of the extra microscope for the preliminary examination of the slides was found to be very convenient, as it permitted the eyepiece camera to be left attached to the research microscope in readiness for the photographic step. This is especially desirable when testing for lignin as the phloroglucin stain is only temporary and fades noticeably within a few minutes.

Very few of the sections tested for fat were photographed as in most cases the fat globules showed up equally well or better in those sections stained for lignin and more contrast was obtained in the photomicrograph.

RESULTS

On the basis of growth habit i.e. ratio of leaf to stem and the anatomical structure, the grasses fall into two fairly distinct groups. Table 1 shows that 95 per cent or more of the dry weight of the native grasses at Stage 4 consisted of leaves. Conversely approximately 90 per cent of the dry weights of the cultivated grasses at Stage 4 consisted of stems. Therefore the most important comparisons will be made between native grass leaves on the one hand and cultivated grass stems on the other.

Table 1

Relative Proportions of Leaf to Stem

in Percentage of Dry Weight

Growth stage	Grass	Leaf	Stem	Head
2	Speargrass	91.0	9.0	
	Fescue*	98.0	2.0	
	Crested wheatgrass	15.0	85.0	
	Brome	24.0	76.0	
3	Speargrass	93.0	7.0	
	Fescue*	98.0	2.0	
	Crested wheatgrass	16.0	66.0	18.0
	Brome	15.0	65.0	20.0
4	Speargrass	96.0	4.0	
	Fescue*	98.0	2.0	
	Crested wheatgrass	13.0	87.0	
	Brome	9.0	91.0	

* Fescue not weighed, estimated only.

With the exception of Stage 5 the results of the analyses of leaf stems and heads reported in Tables 2, 3, 4, and 5, were calculated to a whole plant basis and are reported in Fig. 5, 25, 30, and 31 respectively.

Lignin

The important role that lignin plays in the digestibility of forages has been discussed. Examination of Fig. 8, 14, and 15 shows that lignified tissue in the immature native grass leaf is confined chiefly, if not entirely, to the vascular bundles. Furthermore, in the fescue leaf, Fig. 13, these bundles are smaller in size, and less numerous than they are in the speargrass leaf, Fig. 6, thus constituting a smaller proportion of the total cross sectional leaf area. In addition, Fig. 9 and 16 indicate that as the season progresses, lignin apparently does not infiltrate and encrust the adjacent cellulose fibres to any great extent. Neither is there any sign of lignification in the walls of the mesophyll cells. Biochemical analyses corroborate these observations, the initial lignin content of fescue being much lower than that of speargrass, Fig. 5, and the slope of both curves being similar and relatively flat, indicating that no large increase in lignin content occurred during the season.

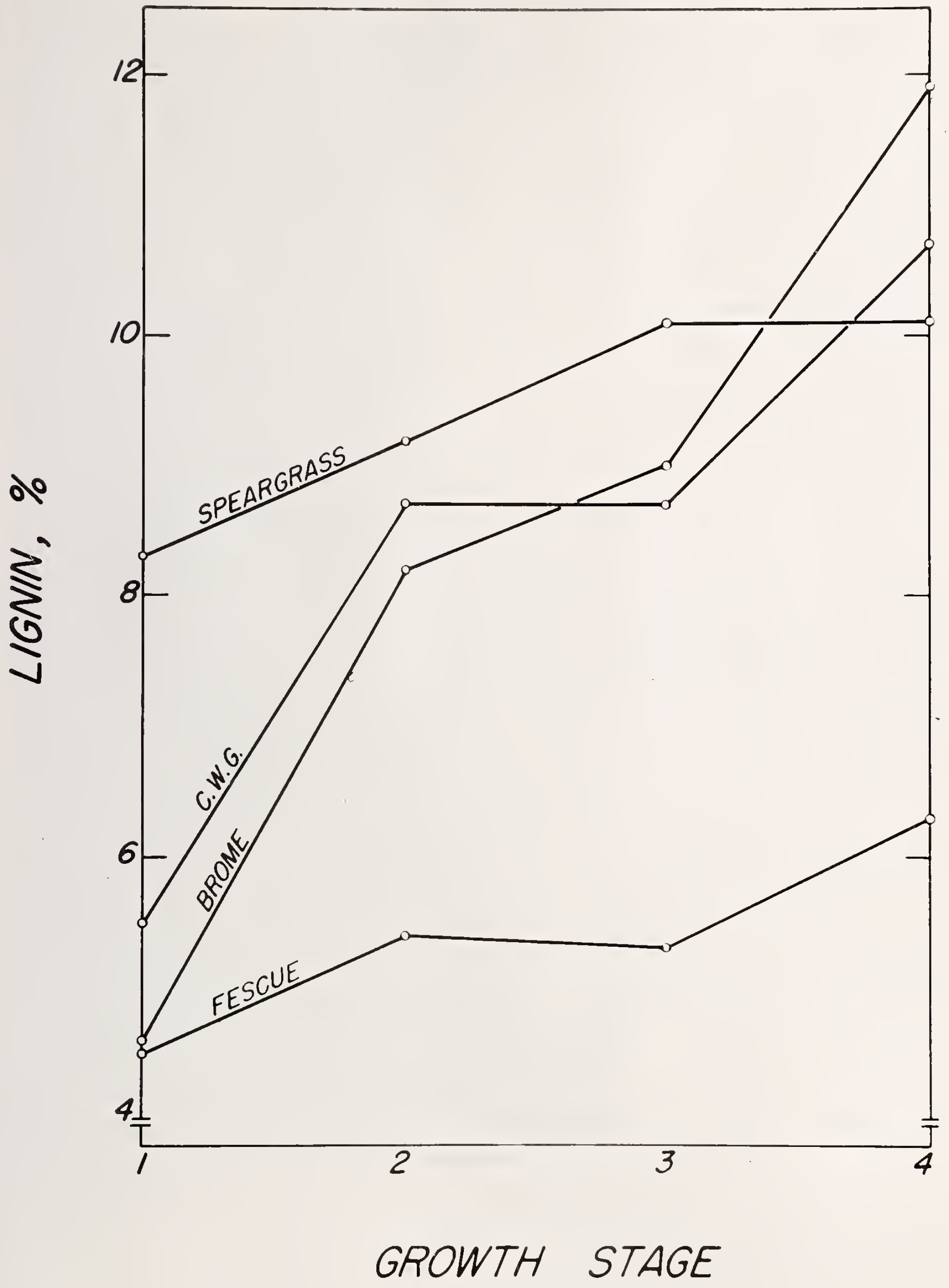

Fig. 5

Effect of Growth Stage on Lignin Content

The two cultivated grasses consist entirely of leaf and stem bases at Stage 1. Fig. 17 and 21 show that here again the major portion of the lignin is confined to the wide-spaced vascular bundles. However, as the plant reaches a more mature stage, there occurs a reversal of the ratio of leaf to stem and there is a preponderance of stem which becomes more marked as the season advances, Table 1.

In the early stages of stem growth, Stage 2, Fig. 19 and 23, the lignification of cell walls was mostly confined to the vascular bundles. At a later growth stage, Stage 4, Fig. 20 and 24, heavy lignification occurred in the walls of the cellulose fibres and the parenchyma adjacent to the vascular bundles. A very high proportion of the cross sectional area of the stem tissues was now lignified.

The lignin determinations reflect this relationship. Fig. 5 shows that a very low lignin concentration was present in both cultivated grasses, Stage 1. With advancing maturity a steady rise in percentage took place and the steep slope of both curves indicated a similar trend for the brome and crested wheatgrass.

Fig. 10 shows that the stem and leaf sheath of speargrass consisted chiefly of structural material. At Stage 2 there was little lignification of the fibres and parenchyma.

At Stage 4 (not illustrated) the fibres and parenchyma were both heavily lignified. This agrees with the high lignin values reported for speargrass stem at Stage 4, Table 2.

The histological observation on the progress of lignification in the cultivated grasses are similar to those reported by Drapala _et_ _al_ (15) for red clover.

Common speargrass

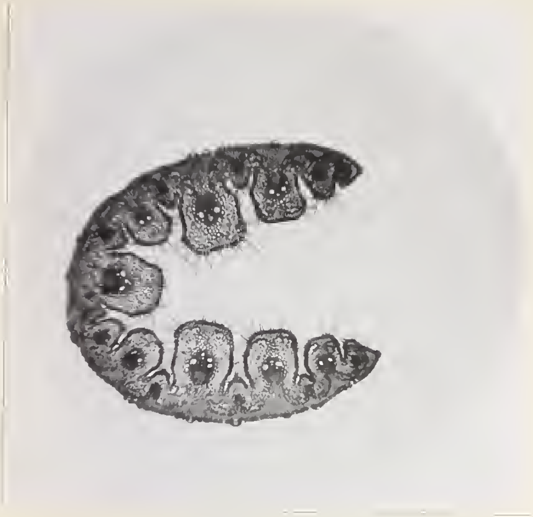

Fig. 6 (x 40)

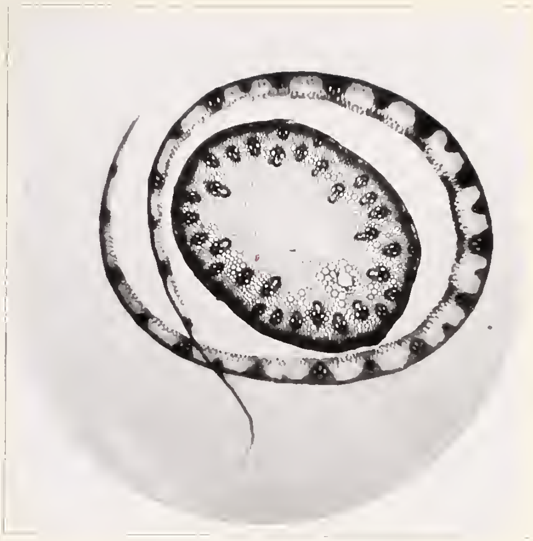

Fig. 7 (x 20)

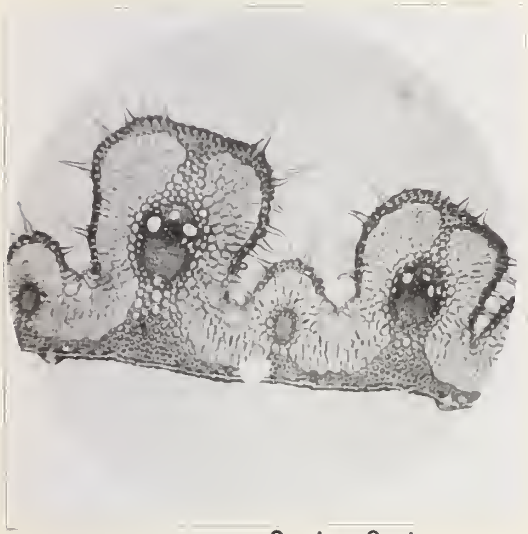

Fig. 8 (x 80)

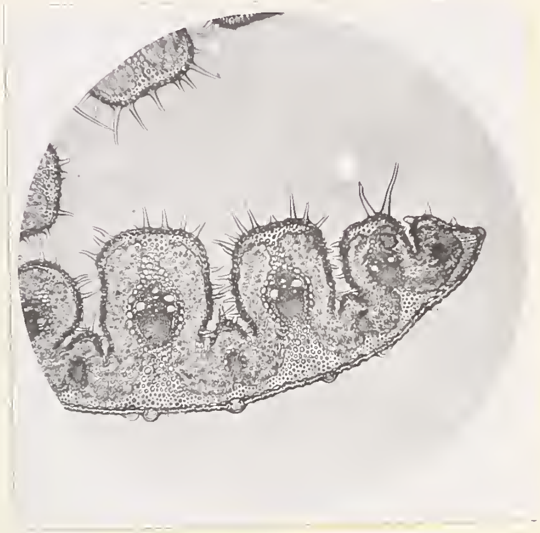

Fig. 9 (x 80)

Cross Sections of Leaf and Stem

Fig. 6 (Stage 4). General outline of rolled leaf.

Fig. 7 (Stage 2). General outline of stem and leaf sheath.

Fig. 8 (Stage 2). Basal leaf. Fibre development not yet complete. No lignification of fibres.

Fig. 9 (Stage 4). Basal leaf. Fibre development complete; some lignification in fibres especially below vascular bundles. Fat globules visible in mesophyll cells.

Common speargrass

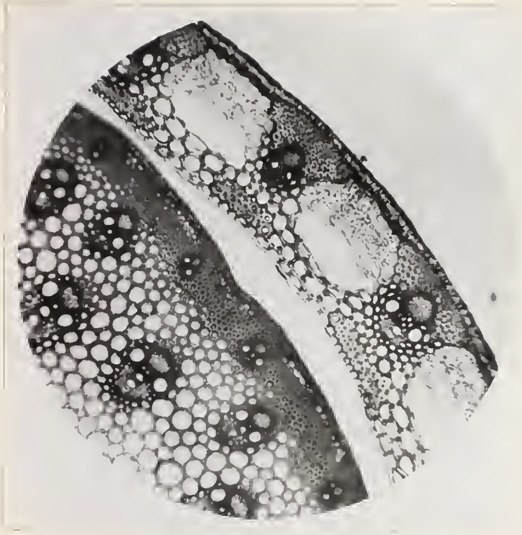

Fig. 10 (x 80)

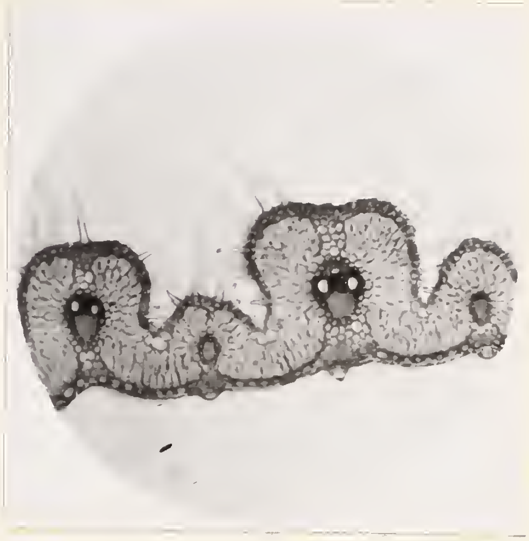

Fig. 11 (x 80)

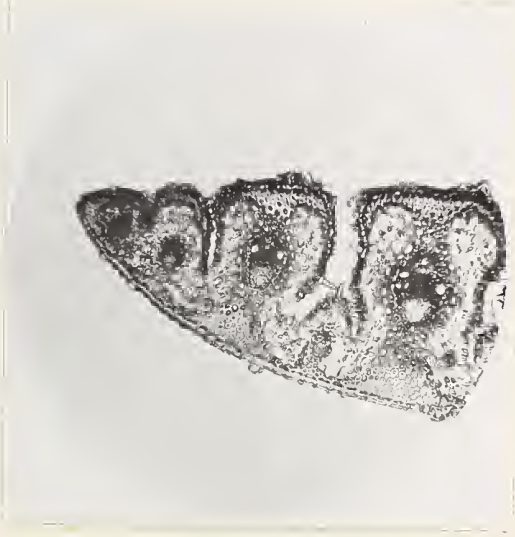

Fig. 12 (x 80)

Cross Sections of Leaf and Stem

Fig. 10 (Stage 2). Detail of stem and leaf sheath.

Fig. 12 (Stage 3). Basal leaf showing disintegration of the mesophyll; general form and structure of the leaf maintained by supporting fibres.

Fig. 11 (Stage 2). Stem leaf similar to basal leaf but without band of fibres inside lower epidermis.

Rough fescue

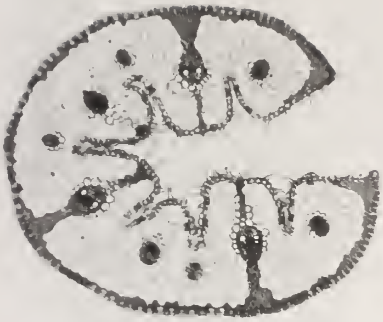

Fig. 13 (x 40)

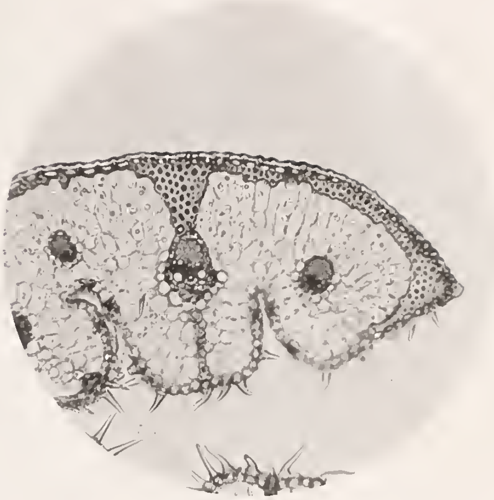

Fig. 14 (x 80)

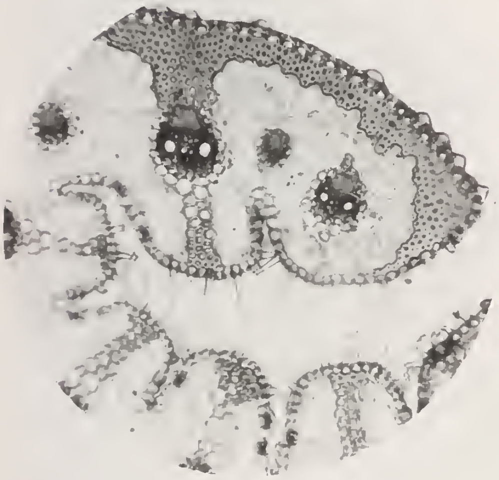

Fig. 15 (x 80)

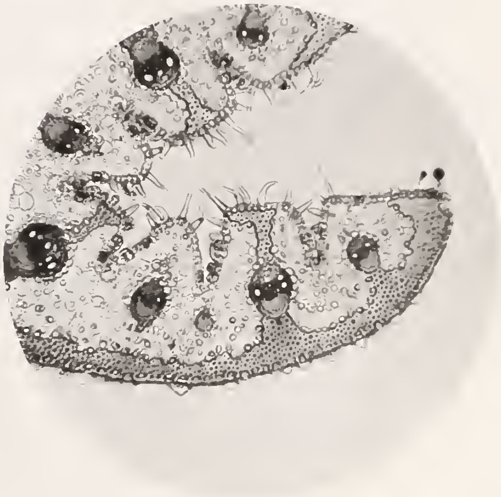

Fig. 16 (x 80)

Figs. 13 to 16. Cross sections of basal leaves showing stages of fibre development and progressive lignification.

Fig. 13 (Stage 1). General outline of permanently rolled leaf.

Fig. 14 (Stage 1). Early fibre development, no lignification.

Fig. 15 (Stage 2). Further fibre development, little or no evidence of lignification.

Fig. 16 (Stage 4). Complete fibre development, slight lignification. Fat globules clearly visible in mesophyll cells.

Crested Wheatgrass

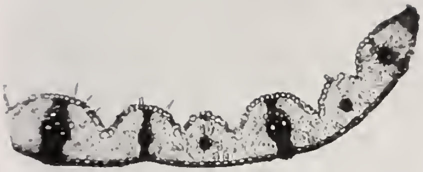

Fig. 17 (x 40)

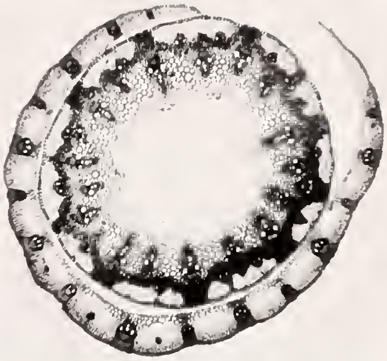

Fig. 18 (x 20)

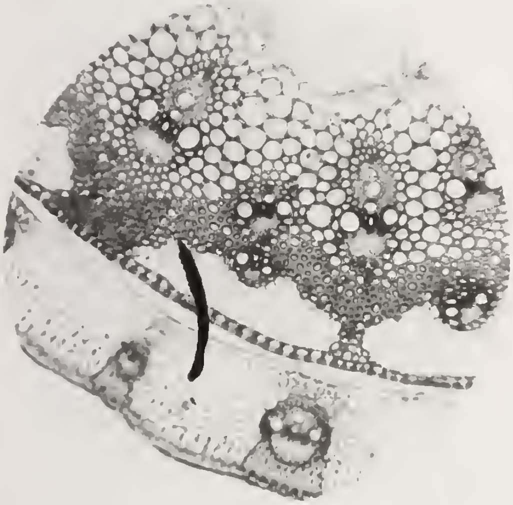

Fig. 19 (x 80)

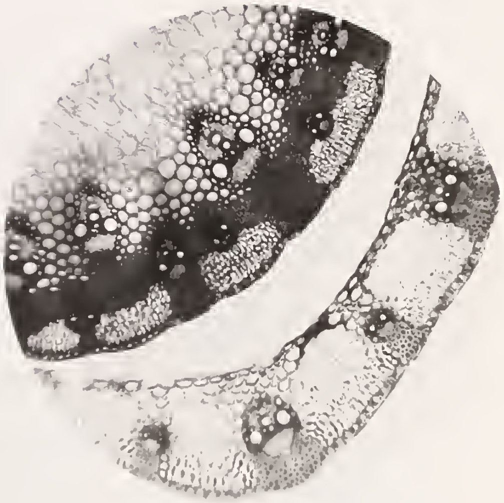

Fig. 20 (x 80)

Cross Sections of Leaf and Stem

Fig. 17 (Stage 1). General outline of a narrow leaf showing leaf structure which is intermediate between brome and native grasses.

Fig. 18 (Stage 2). General outline of stem and leaf sheath.

Fig. 19 (Stage 2). Stem and leaf sheath. Slight lignification of fibres and of stem parenchyma adjacent to the bundles.

Fig. 20 (Stage 4). Stem and leaf sheath. Much heavier lignification of both fibres and stem parenchyma.

Common brome

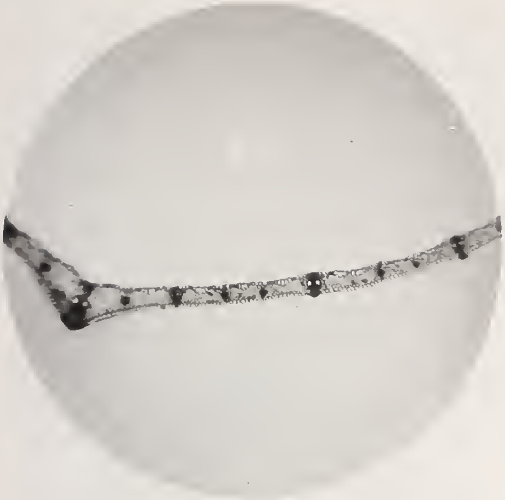

Fig. 21 (x 20)

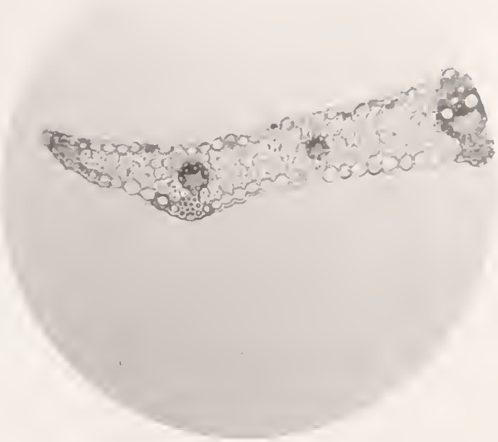

Fig. 22 (x 80)

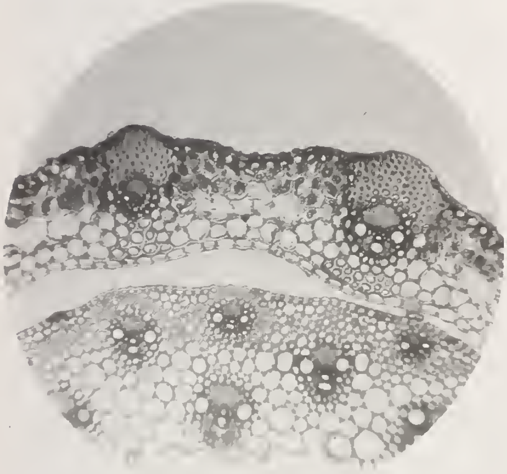

Fig. 23 (x 80)

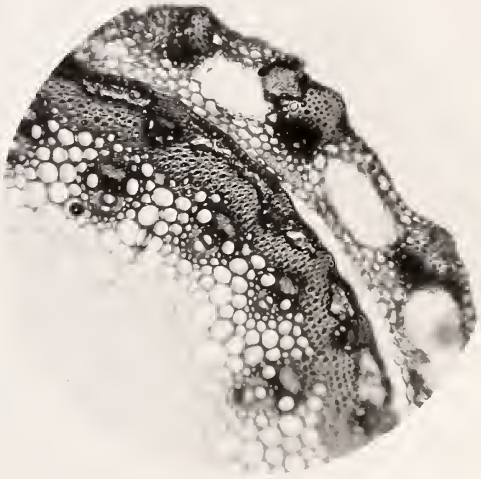

Fig. 24 (x 80)

Cross Sections of Leaf and Stem

Fig. 21 (Stage 1). General outline of a narrow leaf.

Fig. 22 (Stage 1). Enlarged view of leaf margin, Fig. 21, showing widely spaced bundles, few fibres and thin-walled epidermal cells.

Fig. 23 (Stage 2). Stem and leaf sheath showing little or no lignification of fibres but slight lignification of stem parenchyma.

Fig. 24 (Stage 4). Stem and leaf sheath showing much heavier lignification at both fibres and stem parenchyma and disintegration of mesophyll in the leaf sheath.

Table 2

Lignin Content of Four Grasses in Percentage of Dry Weight
(Average of Two Determinations)

	Growth stage	Native		Cultivated	
		Speargrass	Fescue	Crested wheatgrass	Brome
	1	8.3	4.5	5.5	4.6
	2	9.3	5.4	6.3	6.2
Leaf	3	10.0	5.3	8.0	7.9
	4	10.1	6.3	8.6	10.5
	5	9.8	6.9	-	-
	2	9.5	-	9.2	8.8
Stem	3	11.4	-	8.7	9.5
	4	12.0	-	10.9	12.1
Head	3	-	-	9.4	7.8

Cellulose

The success of the curing habit depends basically on the inherent ability of the aerial parts of the plant to resist deterioration over a period of several months following the curing process. The anatomy of the basal leaf of the native grass, Fig. 8 and 15, suggests that reinforcing cellulose fibres play a major role in the observed ability of these leaves to retain their physical form after curing occurs. These fibres form, in most cases, a complete band several fibres wide, around the inside of the lower epidermis, fescue having a heavier band than speargrass. In addition, the vascular bundles are strongly reinforced by fibres extending above and below in the form of "I" beams. The total cross sectional area occupied by the fibres appearsto be somewhat greater in the case of fescue than in that of speargrass, Fig. 9 and 16. This factor agrees with the slightly higher cellulose values reported for fescue leaf than those for speargrass leaf, Fig. 25.

An entirely different picture is presented by the anatomy of the introduced grass leaf. These leaves, brome in particular, Fig. 17 and 21, are wide, thin, less compact, with only a few reinforcing cellulose strands occurring immediately above and below the widely separated vascular bundles, and at the leaf edge, Fig. 21. The weak thin-walled epidermal cells

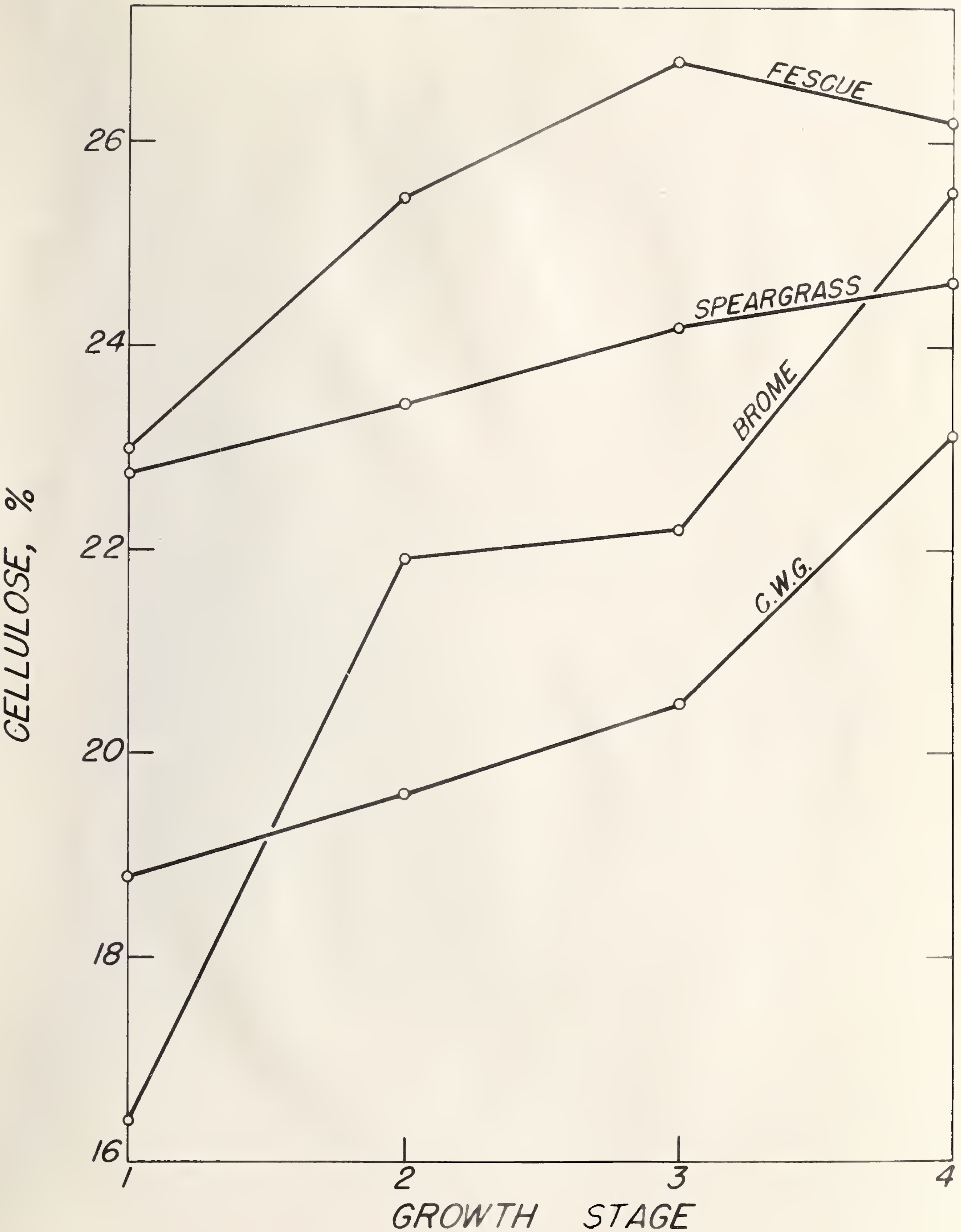

Fig. 25

Effect of Growth Stage on Cellulose Content

of the brome leaf, Fig. 22, should also be observed. Hence the brome leaves are weakly reinforced and this explains why they break up readily soon after they mature in the fall, frequently as early as mid-September. Crested wheatgrass leaves do not lacerate and shred like the brome leaves and this is believed to be due to the fact that they are somewhat narrower, thicker and more compact, Fig. 17. They are much weaker structurally than the native grass leaves, however, and many of these break off after curing takes place. Part of the loss of crested wheatgrass leaves is frequently attributable to grasshopper damage, as well.

Fig. 12 shows that speargrass leaves retain their general form even after long periods of weathering, apparently due to reinforcing fibres.

Additional information on the importance of structural fibres in the leaf was obtained from the two sub-dominants on the speargrass site, namely, sandgrass and ricegrass. Sections were made of both grasses including the early growth, Fig. 26 and 28 and the previous year's growth, Fig. 27 and 29 still standing on the stem.

Fig. 26 shows that sandgrass has a wide, flat leaf similar in outward appearance to the brome leaf. The internal structure, however, is very different in that it has very closely

Sandgrass

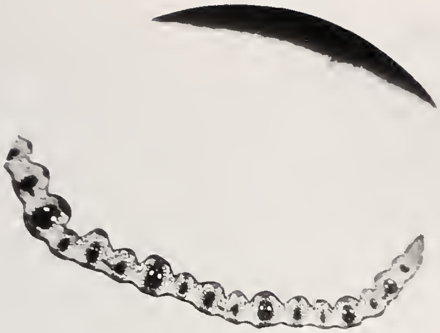

Fig. 26 (x 20)

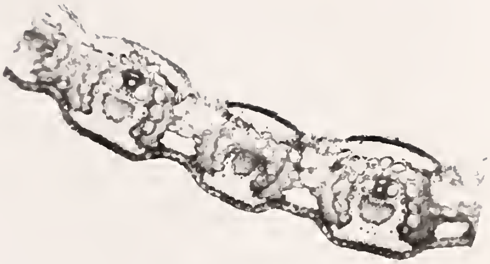

Fig. 27 (x 80)

Indian ricegrass

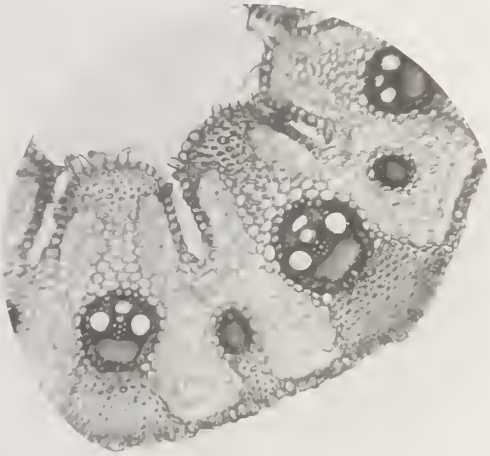

Fig. 28, (x 80)

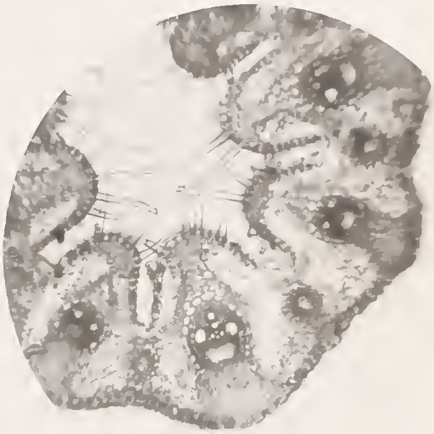

Fig. 29 (x 80)

Cross Sections of Stem Leaves

Fig. 26 (Stage 2). General outline of wide thin leaf showing closely spaced vascular bundles.

Fig. 27 (Stage 5). General form and structure of the leaf maintained by close arrangement of bundles and heavy reinforcement of fibres.

Fig. 28 (Stage 2). Showing leaf structure similar to speargrass, Fig. 12.

Fig. 29 (Stage 5). Showing disintegration of mesophyll but general form and structure maintained by heavy reinforcement of fibres.

spaced vascular bundles which are well reinforced above and below with strands of strong cellulose fibres, Fig. 27, in contrast to the widely spaced, poorly reinforced bundles of the brome leaf, Fig. 21. Although the soft internal tissues of the sandgrass leaf are obviously breaking up at Stage 5 outwardly the old leaf appears to be in an excellent state of preservation with general structure and form well maintained.

It should be mentioned here that this grass is of little value for pasture as it is very tough and unpalatable to livestock. Nevertheless it serves to illustrate the importance of leaf structure in relation to the curing process.

The ricegrass is very closely related to the speargrass (55) and the leaf structures are similar Fig.11 and 28. The old leaf, Stage 5, Fig. 29, shows that the vascular bundles and reinforcing fibres are essentially intact, enabling the leaf to retain its physical form although the softer tissues have largely degenerated. This grass is regarded as a palatable, nutritious forage but it is of little economic importance in the area because of its relative scarcity.

Fig. 25 shows that a much higher proportion of the total cellulose was present in the native grass leaves at early growth stages than was the case for the cultivated grasses. These results are in agreement with the histological observations.

Table 3

Cellulose Content of Four Grasses in Percentage of Dry Weight

(Average of Two Determinations)

	Growth stage	Native		Cultivated	
		Speargrass	Fescue	Crested wheatgrass	Brome
Leaf	1	22.7	23.0	18.8	16.4
	2	23.6	25.5	16.2	15.2
	3	24.2	26.8	18.1	18.1
	4	24.5	26.2	21.2	19.2
Stem	2	22.0	-	20.2	24.1
	3	24.5	-	24.1	26.5
	4	30.2	-	23.4	26.2
Head	3	-	-	10.6	11.7

Ether Extract (Crude fat)

Fig. 30 indicates that the ether extract of both cultivated grasses followed the usual downward trend during the season, while that of speargrass remained nearly constant and that of fescue steadily increased.

Fig. 9 and 16 show that at least part of this increase is due to fat globules which reach substantial proportions particularly in the cells of fescue leaf, Fig. 16 and to a lesser degree in the speargrass leaf, Fig. 9.

As previously mentioned, most of the ether extracts from forages are of low nutritive value. In this instance, Stage 4, the ether extracts of the native grasses were not highly colored as was the case for earlier growth stages so that little chlorophyll was included. Furthermore, ether extracts of native grass leaves, Stage 5, Table 4, showed a relatively high value which shows that this characteristic was not confined to a single season. These observations suggest that possibly much of the crude fat obtained from the native forage, Stage 4, was actually made up of esters of fatty acids. These would be a small but valuable source of energy to the range animal, since true fats have two and one-quarter times the energy value of carbohydrates.

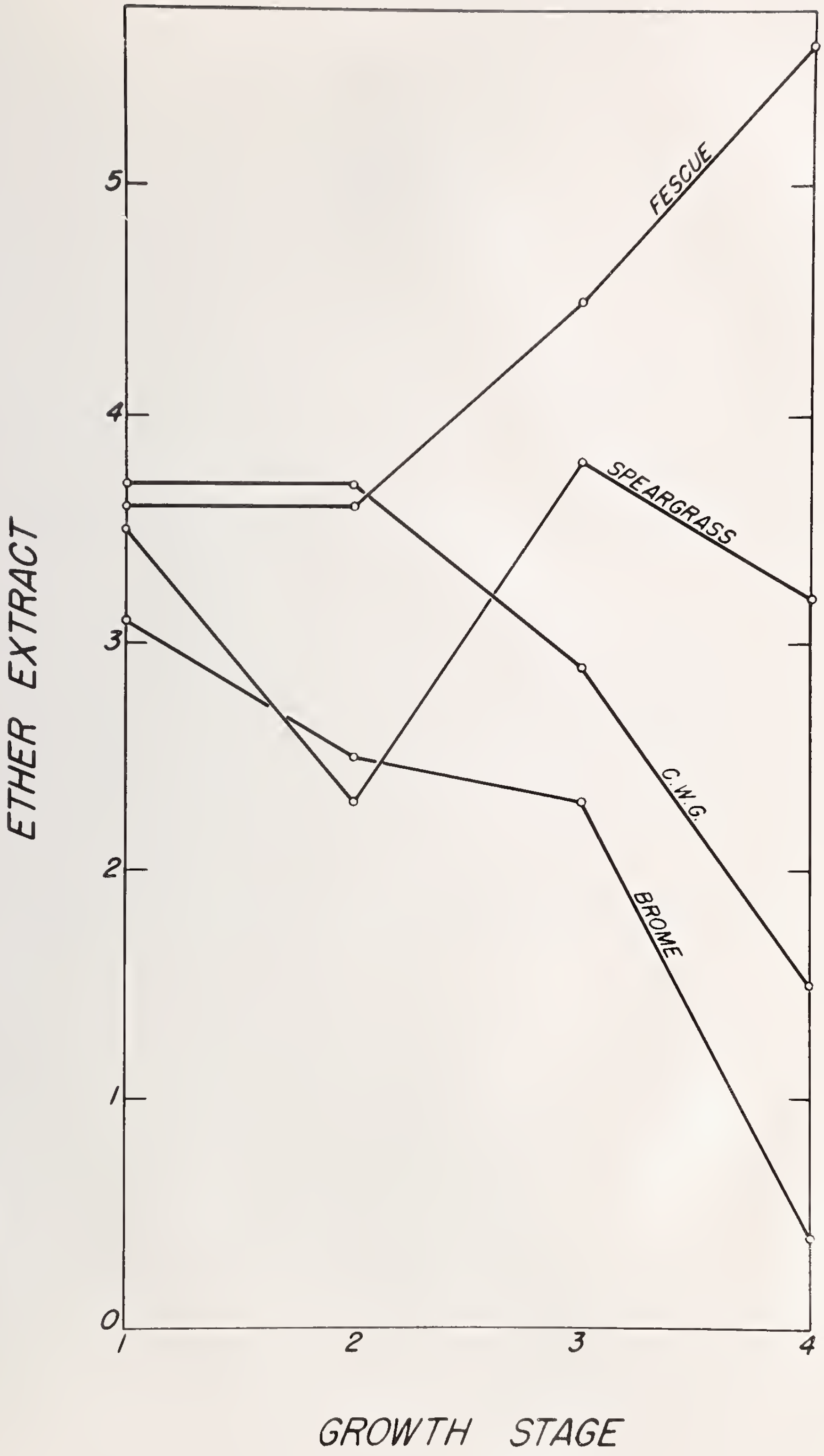

Fig. 30

Effect of Growth Stage on Ether Extract

Table 4

Ether Extract Content of Four Grasses in Percentage of Dry Weight

(Average of Two Determinations)

	Growth stage	Native		Cultivated	
		Speargrass	Fescue	Crested wheatgrass	Brome
Leaf	1	3.5	3.6	3.7	3.1
	2	2.2	3.6	6.8	4.4
	3	3.9	4.5	5.4	4.5
	4	3.3	5.6	3.9	3.0
	5	4.6	3.5	-	-
Stem	2	3.2	-	3.1	1.9
	3	1.8	-	2.2	1.6
	4	1.1	-	1.2	0.1
Head	3	-	-	3.4	2.8

Protein

Fig. 31 shows that there was a steady decline in protein content of all four grasses throughout the season. Speargrass was noticeably higher in protein content than were the other three in the cured state, with crested wheatgrass intermediate and the other two very low. If protein is one of the limiting factors in the digestibility of these roughages, as it may be, then speargrass and crested wheatgrass are sufficiently above the other two to make some difference in nutritive value. Since these data cover only one site for one season, no definite conclusions can be drawn.

Starch

There was no evidence of appreciable amounts of starch at the cured stage in any of the four grasses. Speargrass leaf showed a slightly positive test, but even it did not approach the 1% point.

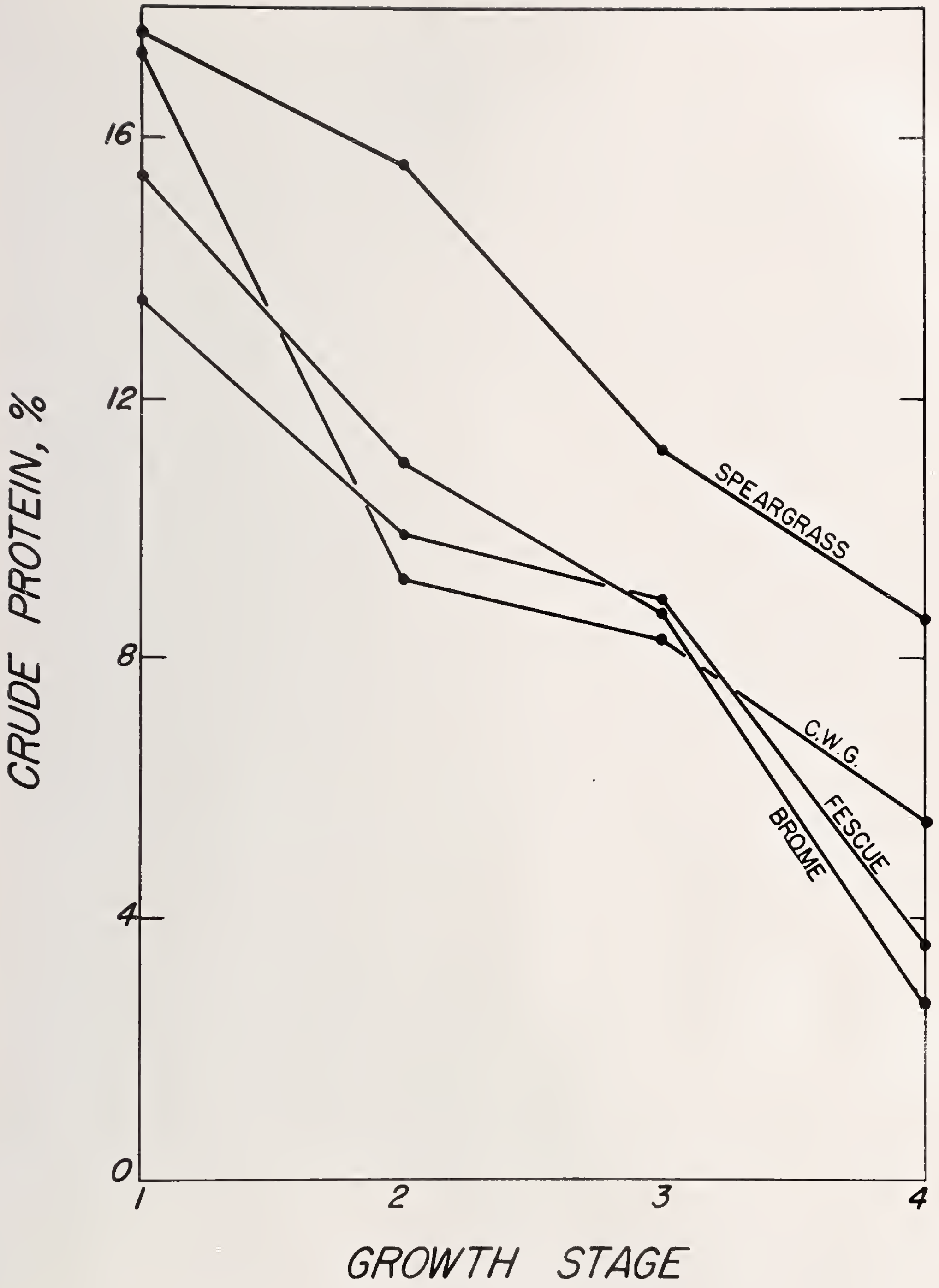

Fig. 31

Effect of Growth Stage on Crude Protein

Table 5

Protein Content of Four Grasses in Percentage of Dry Weight
(Average of Two Determinations)

	Growth stage	Native		Cultivated	
		Speargrass	Fescue	Crested wheatgrass	Brome
Leaf	1	17.6	13.5	17.3	15.4
	2	16.2	9.9	15.2	17.1
	3	11.6	8.9	10.1	14.4
	4	8.8	3.6	6.8	6.4
	5	-	2.6	2.9	-
Stem	2	9.3	-	8.1	9.1
	3	4.9	-	7.0	6.0
	4	4.3	-	5.5	2.3
Head	3	-	-	11.2	13.6

DISCUSSION

Growth was very poor on the speargrass, fescue and crested wheatgrass sites during the 1949 season. The bromegrass site was located in a slightly more favorable position and growth on this area was nearly normal. In addition, some of the late fescue growth was frozen before curing occurred. Whether drought or frost affected the results of the chemical analyses - other than possible hastening lignification (51) - is not known.

The values reported for the lignin analyses of the two cultivated grasses, Stage 4, are considerably lower than those reported for the corresponding grasses in Montana (50, 51). Most of this difference in results is believed to be due to the fact that in that work the so-called formalin procedure for their lignin determination was used. This method was found to give higher apparent lignin values for mature plant material than did the 72 per cent sulphuric acid method (20).

The importance of lignification in the curing process is believed to be not so much total lignin content of the forage but rather the position and extent of the lignified tissues. A large proportion of the native grass leaf, Stage 4, consists of non-lignified mesophyll tissue. Both the cell walls

and cell contents would probably be readily digested by range animals. Also in general, the fibres were not heavily lignified and hence the cellulose in their walls should be quite susceptible to bacterial attack and decomposition in the digestive tract of herbivara.

Conversely, a very high proportion of the stem material of the cultivated grasses is structural material most of which is heavily lignified at Stage 4. This material would probably be digested to a lesser degree than that obtained from the native grass leaves and would provide the animal with less energy.

Further information in this respect is supplied by Patton's (50) observation that blue grama grass, one of the best of the cured native grasses, has a very high lignin content at an early vegetative stage, and a relatively high lignin content in the cured stage. Structurally the leaf of this grass is very similar to sandgrass (60) illustrated in Fig. 26. The numerous and closely-spaced vascular bundles would almost certainly result in a fairly high lignin content but apparently this lignin does not seriously interfere with the utilization of this grass by the range animal.

The crested wheatgrass stems usually stand weathering fairly well, but brome stems are not so resistant and a considerable number break off and blow away during the fall and early winter. This apparent weakness of brome stems may be due to two factors, namely:

(1) The hollow brome stem, Fig. 23, may be weaker than the solid or semi-solid crested wheatgrass stems, Fig. 19 and 20.

(2) The brome stem may be more brittle than that of crested wheatgrass owing to a relatively high lignin content, Table 2. According to Phillips (52) a high lignin content makes plant tissues brittle but the exact concentration required to reach this point is not stated.

The lignin values obtained for speargrass and fescue leaves, Stage 5, are worthy of special comment. They are almost identical with those reported for Stage 4, Table 2. Unless the initial lignin content of these grasses in 1948 was much lower than that of 1949, little losses of soluble constituents have occurred, otherwise the percentage of lignin would have increased.

The results from the dichromate method (16) used for isolating the cellulose agreed well with the progressive formation of fibres in native grass leaves as illustrated in Fig. 9 and 16.

Whether the large increases in cellulose and lignin content of the cultivated grasses, especially brome, are really due to actual increases in lignin and cellulose content between the stages 3 and 4, or are only apparent increases caused by

losses of the more soluble carbohydrates is not known since no chemical analyses were carried out to determine the latter constituents.

With respect to fat, no fatty acid determinations were carried out on the ether extracts so that whether the relatively high values for ether extract obtained from the native grasses, Stages 4 and 5, significantly affect energy value cannot be stated.

Since the results presented in the present studies for each grass are representative of one site and one year, only, further investigations must be carried out along the same lines, possibly with the addition of animal digestion trials before definite conclusions can be reached.

Recommendations for future investigation include the following:

(1) That lignin, cellulose and ether extract be determined on samples of these four grasses, in the cured stage, from several sites for a number of years.

(2) That fatty acid determinations be carried out on the ether extracts from the native grasses.

(3) That soluble carbohydrate determinations be included with the above determinations.

SUMMARY AND CONCLUSIONS

Within the limits of this investigation there is reason to believe that curing is a combination of several factors, the most important of these being:

(1) The position and the extent of the lignified tissues within the plant.

(2) The presence or absence of reinforcing cellulose fibres which enable the plant to withstand weathering over a period of several months.

(3) A relatively high fat content in the cured stage.

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